DISTRIBUTION AND ABUNDANCE OF EXOTIC EARTHWORMS

(OLIGOCHAETA: LUMBRICIDAE) WITHIN THE KENAI NATIONAL WILDLIFE

REFUGE IN SOUTHCENTRAL ALASKA

By

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Abstract:

Exotic earthworms (Oligochaeta: Lumbricidae) have well-documented negative effects on forest ecosystems such as decreasing organic matter in the soil and reducing plant species richness. However, little is known about exotic earthworms in Alaska. This study seeks to document the distribution and possible limitations by soil pH and moisture for exotic earthworms within the Kenai National Wildlife Refuge (KNWR) in southcentral Alaska. We sampled a total of 70 sites near popular fishing areas, along road corridors, and in low human impact areas. Nearly all road sites (90%) and boat launches (80%) contained earthworms, while half (50%) of low human impact sites were occupied. Distance to roads was the only significant factor in predicting earthworm occurrence; soil pH, soil moisture, leaf litter depth, and vegetation cover were not significant predictors. These results suggest that road construction and use, as well as bait abandonment may be mechanisms of earthworm introduction within the KNWR.

Introduction:

Pleistocene glaciations extirpated native earthworms from much of North America, leaving resulting landscapes devoid of earthworms until the introduction of exotic earthworms (Oligochaeta; Lumbricidae) during European settlement (Gates 1970, Gates 1982). The effects of exotic earthworms on forest ecosystems are well documented and vary by feeding strategy. Typically epigeic species, which live within the leaf litter layer are the least destructive as they physically disrupt only the organic layer by consuming and mixing the top layers and producing a homogeneous, coarse form of organic forest litter. Endogeic species live in the top soil (A horizon) and create horizontal burrows within this layer. Anecic earthworms burrow deep into the soil causing them to have the greatest impacts by removing litter from the surface and pulling it down into the mineral layer (Addison et al. 2009). Earthworms can accelerate litter decomposition (Hale et al. 2006, Suarez et al. 2006, Holdsworth et al. 2007a, Addison 2009) and reduce plant species richness (Hale et al. 2006, Holdsworth et al. 2007a). Suarez et al. (2006) found that litter remaining in earthworm-invaded areas in New York was 30-60% less than in reference plots. Holdsworth et al. (2007a) found in a Wisconsin forest that exotic earthworms reduced plant species richness in heavily invaded plots by 17%. Similarly, Hale et al. (2006) documented a negative relationship between exotic earthworm diversity and plant diversity in a Minnesota hardwood forest.

Most studies on exotic earthworms have occurred in temperate regions (Hale et al. 2006, Suarez et al. 2006, Holdsworth et al. 2007a, Addison 2009); less is known about the distribution and effects of earthworms in boreal forests (Cameron et al. 2007, Cameron and Bayne 2009). In northern Alberta, Cameron et al. (2007) found boat

launches and roads had a higher probability of earthworm occurrence than other locations. Their results suggested vehicle transport and bait abandonment as primary mechanisms of earthworm introduction.

As for most invasive species, human activities, particularly road construction and unintentional transport, likely increase the rate of spread for exotic earthworms above their natural dispersal of 5-10 meters a year (Gundale et al. 2005, Addison 2009). Consequently exotic earthworms are more likely to occur near roads due to availability of disturbed habitats caused by road construction and maintenance that allow for potential establishment, as well as the creation of dispersal corridors (Cameron and Bayne 2009). Vehicles themselves function as vectors transporting earthworm cocoons, which are sticky, mucus coated sacks containing developing embryos (Barnes et al. 2001, Gundale et al. 2005). Several species such as *Lumbricus terrestris* (anecic) and *L. rubellus* (epi-endogeic) are sold commercially as fishing bait and possibly introduced into ecosystems where anglers discard unused bait (Cameron et al. 2007).

There are limited studies on exotic earthworms in Alaska; however, occurrence has been documented for 17 species (Berman and Marusik 1994, Costello et al. 2010, Bowser 2010). Berman and Marusik (1994) collected earthworms between Anchorage and Fairbanks in the litter of a mixed birch-spruce forest, and found numerous epigeic *Dendrobaena octaedra*. Costello et al. (2010) found eight exotic earthworm species in riparian zones on Prince of Wales Island. Bowser (2010), based on limited spatial sampling, found the most abundant species on KWNR was *D. octaedra*.

Factors such as pH and temperature likely limit earthworm distribution, especially in Alaska. Earthworms are usually found in areas with pH of 5--7.4. *Dendrobaena*

octaedra have been found in areas with a pH as low as 2.8--3.6 and *L. rubellus* has been found in areas with pH as low as 3.0 (Addison 2009). Most earthworms are fairly intolerant of freezing, suggesting that winter temperatures may limit range. Survival in low temperature areas depends on the species, stage of development, and other factors. For example, *D. octaedra* is extremely frost tolerant and can withstand freezing in all stages of development (Addison 2009). Holmstrup et al. (2007) found that populations of *D. octaedra* from various geographical areas were able to tolerate freezing down to at least -14ºC, the coldest temperature used in their experiment.

In addition to increasing human impact on the landscape, a rapidly warming and drying climate in Alaska is likely improving environmental conditions for earthworms. Wetlands in Alaska, particularly in the KNWR, are warming and drying (Klein et al. 2005, Riordan et al. 2006, Berg et al. 2009) and average winter temperatures have warmed 3.8--5 °C since 1977 (U.S. Fish and Wildlife Service [FWS], 2010). These drying wetlands and warmer winters may provide more suitable habitat for exotic earthworms. Addison (2009) suggested that even small increases in winter temperatures will lead to large increases in earthworm habitat.

The present study seeks to document species composition, distribution, and habitat correlates for earthworms in the KNWR, particularly in light of a changing climate. A secondary goal was to determine relationships between earthworm presence\absence and distance from human-disturbed areas, such as roads and popular fishing areas. The final goal was to identify possible factors limiting earthworm distribution, such as low pH and varying soil moisture levels. These factors are the ones most likely to change across the Kenai as the climate continues to warm and dry.

Methods:

Study area:

Located on the Kenai Peninsula in southcentral Alaska, USA (60° N, 150° W), the Kenai National Wildlife Refuge (KNWR) is over 777,000 ha with 35% accessible by road. Mountains and glaciers occupy the southeastern KNWR (Figure 1). Bordered by Cook Inlet to the west, and the Kenai Mountains to the east, the Kenai Lowlands portion of the KNWR is one of few areas in Alaska free of permafrost. The Kenai Lowlands are mantled by glacial deposits that vary in texture and are capped by silt loam derived from post-glacial windblown loess. Sloped areas are vulnerable to erosion, especially if the vegetation cover is removed (FWS, 2010). The Lowlands consist of wetlands and mixed boreal forest (Klein et al. 2005) dominated by black spruce (*Picea mariana*), white spruce (*Picea glauca*), birch (*Betula* spp.), and quaking aspen (*Populus tremuloides*). The climate of the Kenai Peninsula is subarctic with a maritime influence. Temperatures rarely rise above 26 °C in summer or drop below -18 °C in winter. The frost-free growing season varies from 71 to 129 days depending on location. The Refuge receives about 480 mm of total precipitation per year (FWS, 2010).

Over 130 historic cabins have been inventoried on the Refuge along with other historic resources associated with mining, trapping, oil development, and road construction. Mining and fishing in the area appeared in the late 1800s and early 1900s, and oil exploration began in the northern part of the Peninsula in the Swanson River area in the mid-1950s. Fire has also been prevalent within the Refuge in the past. Major fires of unknown origin occurred in 1871, 1883, 1891, and 1910. Two large human-caused fires occurred in 1947 (burned 125,000 hectares) and 1969 (burned 35,000 hectares) that

resulted in replacement of mature spruce forests by a mosaic of young forest in various stages of succession. There are 2,900 km of seismic lines which were bulldozed as early as the 1950s and 60s, mainly in the northern part of the Refuge. There are also 240 km of utility and transmission lines and 180 miles of established trails within the Refuge (FWS, 2010). All of these historic disturbances provide past opportunities for earthworm introduction and establishment on the KNWR.

Experimental design:

Earthworms were sampled throughout the KNWR during July and August 2011 at 70 total sites representing three levels of human impact. These levels of human impact were characterized by explicit vectors of introduction, (1) boat launches ($n = 20$), (2) road corridors ($n = 20$) and non-explicit vectors, (3) low impact areas (> 600 m from any road or facility and 50 m from any trail or river; $n = 30$). The sampling site locations were chosen within a GIS (ArcGIS v10) by first identifying suitable areas for each impact level and then randomly selecting sample sites.

Three 0.25 m^2 plots were established at each site to sample earthworm presence. At road and boat launch sites, plots were placed two, three, and five meters from the road or edge of a boat launch. The plots were located approximately ten meters apart when possible, establishing a wider area for detecting earthworm presence.

Plot level sampling:

We sampled earthworm abundance at each plot using a 0.25 m^2 quadrat. Within each quadrat we removed and hand-sorted surface organic material for earthworms. We extracted additional earthworms with a liquid mustard solution (40 g ground mustard powder in 3.8 L water). Earthworms were collected and stored in 70% ethanol.

We measured leaf litter depth by clearing a small area and measuring the vertical depth of the leaf layer with a 30 cm ruler within each plot. Soil pH, (Soil pH Meter, HANNA, RI), and moisture (Digital Moisture Meter, General, NY) were measured in the field. In each plot, we estimated the percentage cover of litter, grass, forbs, moss, and lichen. General forest type (deciduous, mixed, conifer) of the area was determined from field observations and a GIS land cover layer.

We identified collected earthworms to species level when possible based on visual observations of external morphology (Reynolds 1977). An additional 13 earthworms, collected at geographically remote sites, were genetically identified to species level using COI gene to confirm taxonomic identification. Juveniles were grouped into two categories: (1) *Lumbricus* spp. and (2) *other* immature. We estimated ash-free dry biomass (g) from the length (mm) of each preserved specimen with the allometric equation of Hale et al. (2004):

$$biomass = \exp[2.2853 \times \ln(length) - 11.9047]$$

Hale et al. (2004) found that the allometric equations for *Aporrectodea* spp., *Lumbricus* spp., and *Dendrobaena octaedra* were not significantly different from one another, allowing one equation for all species. The fresh weight of an earthworm can vary up to 40% depending on soil moisture and gut contents (Lee 1985), but using the lengths can give comparable results to directly determining the ash-free dry biomass (Hale et al. 2004).

Lumbricus spp. distribution sampling:

Because the anecic *Lumbricus* species have been identified as potentially more damaging than other genera (Eisenhauer et al. 2007), at sites where *Lumbricus* spp. were

identified, additional sampling with three transects estimated the extent of local

distribution. One transect was created perpendicular to the site and the other two at

approximately 45 degree angles from the site. At ten meter intervals along each transect

we sampled three plots for earthworm presence using liquid mustard extraction within a

0.25 m^2 quadrat. Earthworms were collected and stored in 70% ethanol, and later

identified in the lab. We continued along each transect until we found two consecutive

ten meter intervals with all three plots absent of earthworms   Since all transects at

individual sites ended within ten meters of one another, this appeared to be an accurate

indication of the limit of *Lumbricus* spp. in each area.

Statistical analysis:

Individual plots served as replicates for each site, but we used site level data for

most analyses by averaging plot level data. A site was determined to contain earthworms

if individuals were detected in at least one plot. We used Program PRESENCE (version

3.1, J. E. Hines, USGS, Laurel, MD) to compare the sampled proportion of area occupied

to the estimation of area occupied based on the plot replicates. MacKenzie et al. (2002)

presented this model for estimating the site occupancy probability for a target species in

situations where the species is not guaranteed to be detected even when present at the

site.

Independence between earthworm presence and human impact level (road, boat

launch, low impact) and vegetation type (mixed, conifer, open) was assessed using a chi-

square test of independence. We used MANOVA to determine the effect of the factors

impact level and *earthworm occurrence* on the three dependent variables of *soil pH*, *soil*

moisture (%), and *leaf litter depth (cm)*. Soil moisture and leaf litter depth were square-

root transformed for normality. Arithmetic (untransformed) mean and standard errors are reported.

We calculated remoteness for each site as a measure of distance from nearest road. This distance, Y (km), was calculated in GIS by using true surface distance, as it accounted for elevation changes and also masked out lakes. To approximate a normal distribution for analyses, we transformed this distance using $\ln (Y + 1)$.

A principal component analysis (PCA) was performed using all nine scale variables collected at each plot and averaged for each site (soil pH, soil moisture, leaf litter depth, path distance, and moss, grass, lichen, litter, and forbs cover percentages) to reduce the dimensionality of the data set. Results of the PCA were used in a binary logistic regression performed in *R* (version 2.13.2) to predict presence and absence of earthworms. The chi-square, MANOVA, ANOVA and PCA statistical tests were performed using SPSS statistical software (version 19, IBM, Armonk, NY). All statistical tests were considered significant at $p \leq 0.05$.

Results:

Distribution and abundance:

Our survey of the Kenai National Wildlife Refuge (KNWR) for exotic earthworms revealed four earthworm species: *Dendrobaena octaedra*, *D. attemsi*, *Dendrodrilus rubidus*, and *Lumbricus terrestris* among three levels of human impact (road, boat launches, and low disturbance). No site had all four species present. *D. octaedra* was the most widespread and showed no localized pattern in its distribution. *L. terrestris,* found at three sites, was localized to the Swan Lake Road in the northern

region of the KNWR. *Dd. rubidus* was also found in three sites, geographically distant from one another. *D. attemsi* was present at two sites located near Tustumena Lake. The 13 individuals that were genetically identified using COI gene showed > 96% probability of identity to their respective species based on the Basic Local Alignment Search Tool (BLAST) within GenBank. The program compares nucleotide sequences to sequence databases and calculates the statistical significance of matches. Results indicated that all of these individuals were exotic to Alaska because they shared genotypes with other individuals in North America. Overall, among the 70 sample sites, the frequency of sites occupied by earthworms was 70%, a figure very similar to the calculated PRESENCE model estimate of (± standard error) 72.2 ± 0.06% of our sites should contain earthworms. Our detection probability was 0.67 ± 0.044% or the chance of finding earthworms at each individual plot. Nearly all road sites (90% of 20 total sites) had earthworms in at least one plot as did most boat launches (80% of 20 total sites). In contrast, only half (50% of 30 total sites) of the low impact sites contained earthworms. *D. octaedra* was the most widespread adult species, found at 33 sites; *Lumbricus* spp. and *Dd. rubidus* were both found at three sites. *D. attemsi* was found at two sites and the remaining eleven sites contained other, unidentified species of immature earthworms (Figure 1). Three sites (two boat launch, one road) contained at least two species of earthworms (not including immature individuals due to unknown species), while the majority of sites contained only one species (in addition to other possible species as immatures; Figure 2). Immature earthworms that were not *Lumbricus* spp, were likely *Dendrobaena octaedra*.

Lumbricus spp. occurred at three sites, all of which were boat launches located adjacent to one another on Swan Lake road in the northern part of the Refuge (Figure 1). The local distribution was further sampled as it appeared to be a restricted and new introduction. *Lumbricus* spp. had dispersed the farthest at Fish Lake where earthworms were found 110 m from the boat launch. Earthworms occurred 30 m and 40 m respectively, from the Swan Lake west canoe entrance and Merganser Lake boat launch. All other physical and biological variables were similar at these boat launches to other areas within the Refuge.
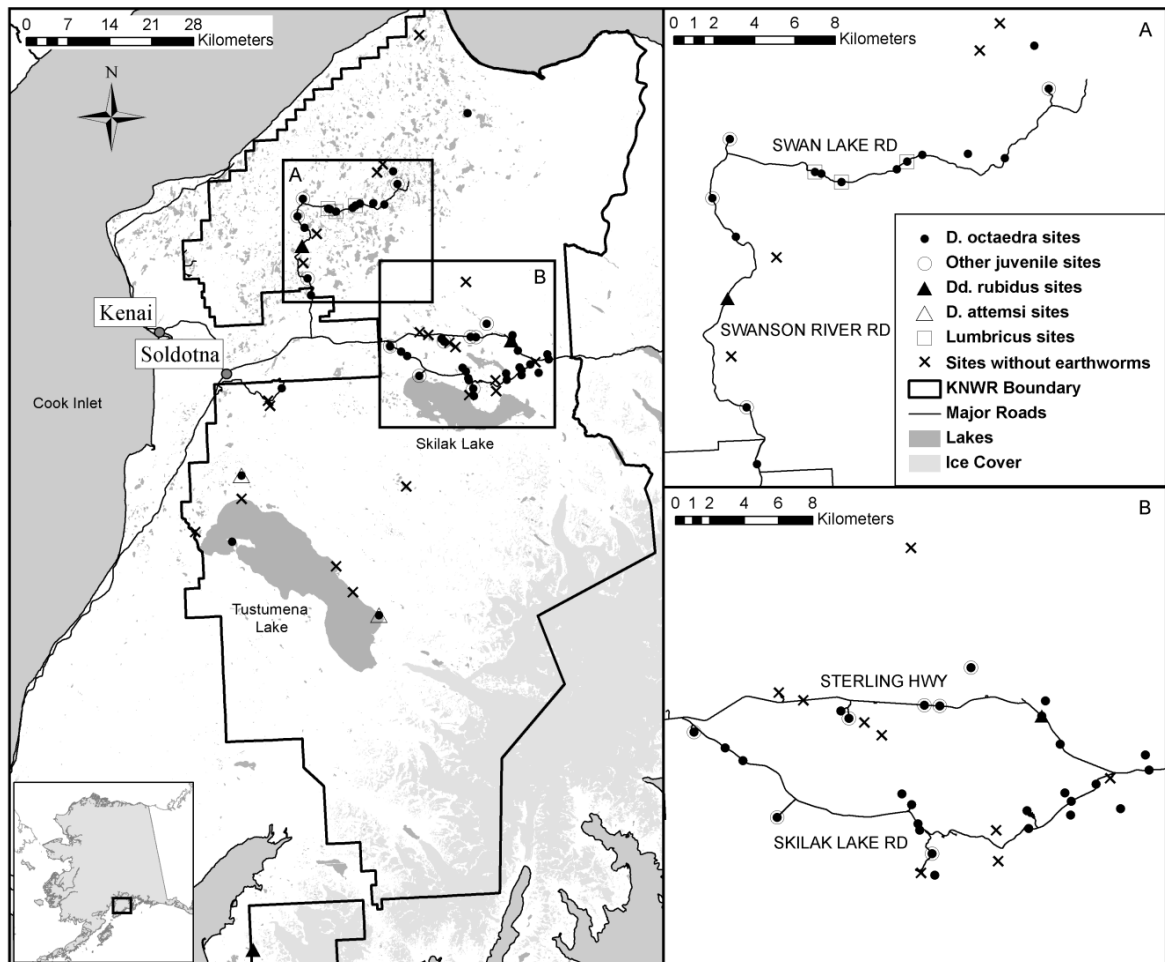

Figure 1. Earthworm species locations for 70 sample sites within the Kenai National Wildlife Refuge. Inset A includes active oil development in the Swanson River Oilfields, and Swan Lakes Canoe Trail. Inset B includes Skilak Lake recreational area and campgrounds.
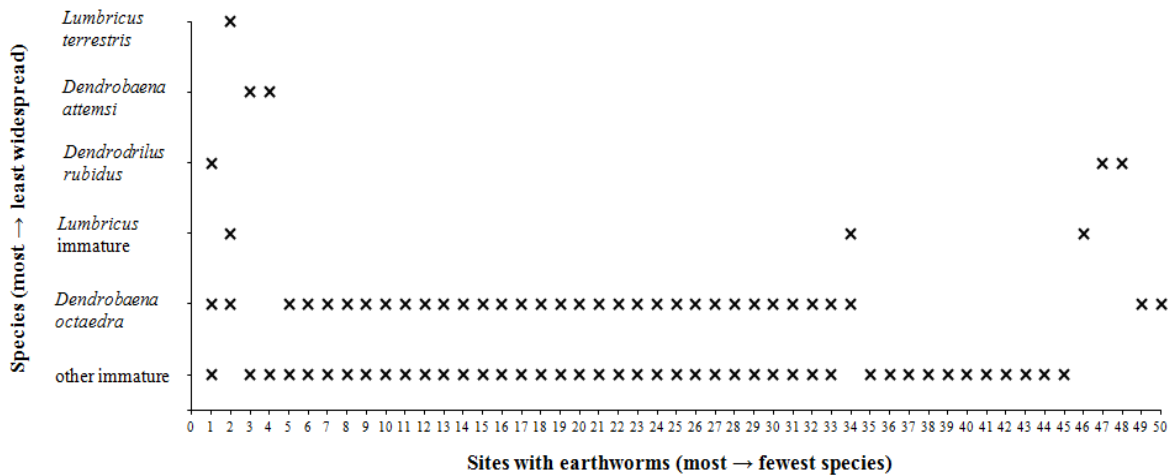
Figure 2. Earthworm species distribution by site for sites containing earthworms.

Earthworm densities showed substantial variation (0--272 ind./m^2; Figure 3). Where present, the mean density of earthworms over all sites was 41 ± 6.1 ind./m^2 (Table 1) and highest at boat launches (mean ± se, n: 50.0 ± 9.0 ind./m^2, n = 16). Low impact sites averaged four more earthworms per meter squared (39.5 ± 17.1 ind./m^2, n = 15) than road sites (35.0 ± 6.7 ind./m^2, n = 18); however, mean density did not differ significantly among impact levels (oneway ANOVA: $F_{2, 46}$ = 0.481, p = 0.621). Earthworm biomass also showed moderate variation (0.31 ± 0.12 AFDg/m^2, n = 49; Figure 4). As with density, boat launches had the greatest biomass (Table 2) of earthworms [0.68 ± 0.35 ash-free dry grams (AFDg)/m^2, n = 16], roads had the lowest (0.11 ± 0.03 AFDg/m^2, n = 18), and low impact sites were intermediate. The mean earthworm biomass was marginally significantly different among impact levels (oneway ANOVA: $F_{2, 46}$ = 2.445, p = 0.098). In summary, boat launches supported both the most biomass and highest number of earthworms, whereas roads had both the smallest biomass and fewest earthworms.

Habitat variables and earthworm occurrence:

Earthworms occurred more frequently at roads and boat launches sites than expected, but much less frequently at low impact sites than expected (Table 3), and their occurrence was not independent of impact level (chi-square test of independence: ($x^2 =$ 11.18, p = 0.004, df = 2, *n* = 69). Similarly, mixed forest and open areas had more sites with earthworms than expected, conifer sites had fewer sites with earthworms than expected (Table 4) and earthworm presence was not independent of site vegetation type ($x^2 =$ 6.83, p = 0.03, df = 2, *n* = 69).

We investigated the effect of the two factors *impact level* and *earthworm occurrence* on the three dependent variables of *soil pH*, *soil moisture*, and *leaf litter depth* using two-way MANOVA. Box's test for homogeneity of variance-covariance was significant ($F_{24,914} = 2.22$, p = 0.001), indicating that variances differed (probably due to unequal sample sizes), so the more robust Pilliai's trace test statistic was used for interpreting MANOVA results (Mertler and Vannatta 2010). The two-way MANOVA
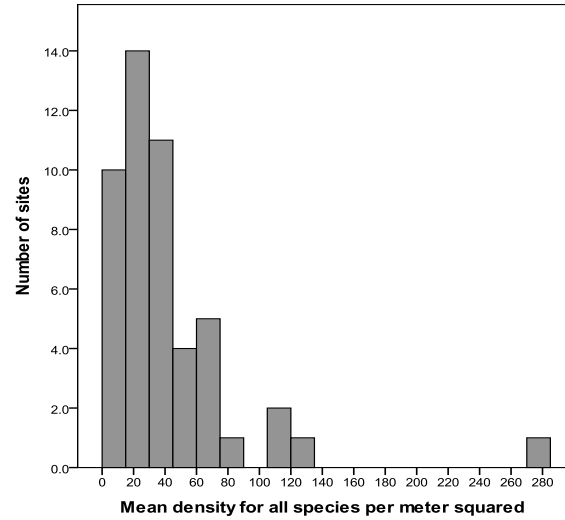

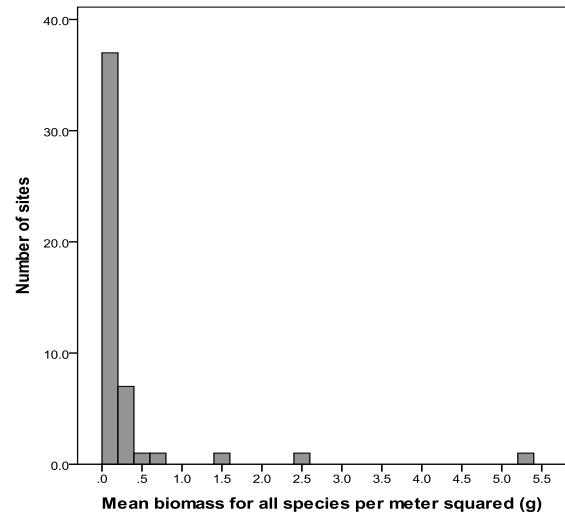Figure 3. Distribution of mean earthworm density across sites containing earthworms (*n* = 49).

Figure 4. Distribution of mean biomass across sites containing earthworms (*n* = 49).

Table 1: Mean densities (individuals extracted/m^2) and standard errors of earthworm species where observed at boat launch, road, and low impact sites.

Site type	*Dendrobaena octaedra*	*Dendrobaena attemsi*	*Dendrodrilus rubidus*	*Lumbricus terrestris*	*Lumbricus* immature	Other immature	Total density
Boat launch (*n* = 16)	5.0 ± 1.6	-	-	0.7 ± 0.7	11.7 ± 6.3	32.7 ± 8.8	50.0 ± 9.0
Road (*n* = 18)	9.2 ± 2.1	-	1.2 ± 0.8	-	-	24.6 ± 4.8	35.0 ± 6.7
Low impact(*n* = 15)	16.7 ± 6.4	1.8 ± 1.2	1.1 ± 1.1	-	-	19.9 ± 11.3	39.5 ± 17.1
Total (*n* = 49)	10.1 ± 2.2	0.5 ± 0.4	0.8 ± 0.4	0.2 ± 0.2	3.8 ± 2.2	25.8 ± 4.8	-

Table 2: Mean biomass (mg/m^2) and standard deviations of each earthworm species where observed by human impact level.

Site type	*Dendrobaena octaedra*	*Dendrobaena attemsi*	*Dendrodrilus rubidus*	*Lumbricus terrestris*	*Lumbricus* immature	Other immature	All species
Boat launch (*n* = 16)	30 ± 39	-	-	286 ± 1144	287 ± 710	84 ± 110	675 ± 1400
Road (*n* = 18)	64 ± 98	-	4 ± 13	-	-	64 ± 70	109 ± 122
Low impact(*n* = 15)	110 ± 136	7 ± 18	3 ± 13	-	-	48 ± 75	158 ± 192
Total (*n* = 49)	67 ± 101	2 ± 10	3 ± 11	93 ± 654	94 ± 420	65 ± 86	-

indicated no interaction between impact level and earthworm occurrence on the dependent variables (Pilliai's trace = 0.106, $F_{6,124}$= 1.15, p = 0.335). The combined dependent variables differed among site types (Pilliai's trace = 0.442, $F_{6,124}$= 5.86, p < 0.001), whereas the combined variables did not differ between earthworm present and absent sites (Pilliai's trace = 0.028, $F_{3,61}$= 0.58, p = 0.628).

Follow-up univariate ANOVA and Tukey post hoc tests showed road sites had the highest soil pH (6.24 ± 0.09, *n* = 20) and low impact sites the lowest (4.92 ± 0.18, *n* = 29). Low impact sites were significantly different in pH (Figure 5) from both road and boat launch sites ($F_{2, 66}$ = 22.12, p < 0.001, Tukey post hoc: p < 0.001). Boat launches had double the soil moisture (7.2 ± 0.75%, *n* = 20) of road sites (3.2 ± 0.72%, *n* = 20). Boat launches were significantly different in soil moisture (Figure 5) from both roads and low

impact sites (ANOVA: $F_{2, 66} = 8.78$, p < 0.001, Tukey post hoc: p < 0.001, p = 0.008, respectively). Road sites had the deepest leaf litter thickness (2.3 ± 0.34 cm, $n = 20$) while low impact sites had the shallowest

Table 3: Observed and (expected) values for earthworm occurrence in boat launch, road, and low impact sites from a chi-square test of independence.

	Site type		
	Boat launch	Road	Low impact
Earthworms present	16 (13.9)	18 (13.9)	14 (20.2)
Earthworms absent	4 (6.1)	2 (6.1)	15 (8.8)

Table 4: Observed and (expected) values for earthworm occurrence at sites in conifer forests, mixed forests, and open areas from a chi-square test of independence.

	Site vegetation type		
	Conifer	Mixed	Open
Earthworms present	4 (7.7)	21 (19.5)	23 (20.9)
Earthworms absent	7 (3.3)	7 (8.5)	7 (9.1)

(1.3 ± 0.31 cm, $n = 29$). The difference between road and low impact sites was significant (ANOVA: $F_{2, 66} = 5.244$, p = 0.008, Tukey post hoc: p = 0.006; Figure 5). In summary, while plot level variables differed among impact levels (boat launch, road, low disturbance), they did not vary between sites with and without earthworms.

PCA and logistic regression:

A principal components analysis (PCA) reduced the dimensionality of nine independent variables (soil pH, soil moisture, leaf litter depth, path distance, and moss,
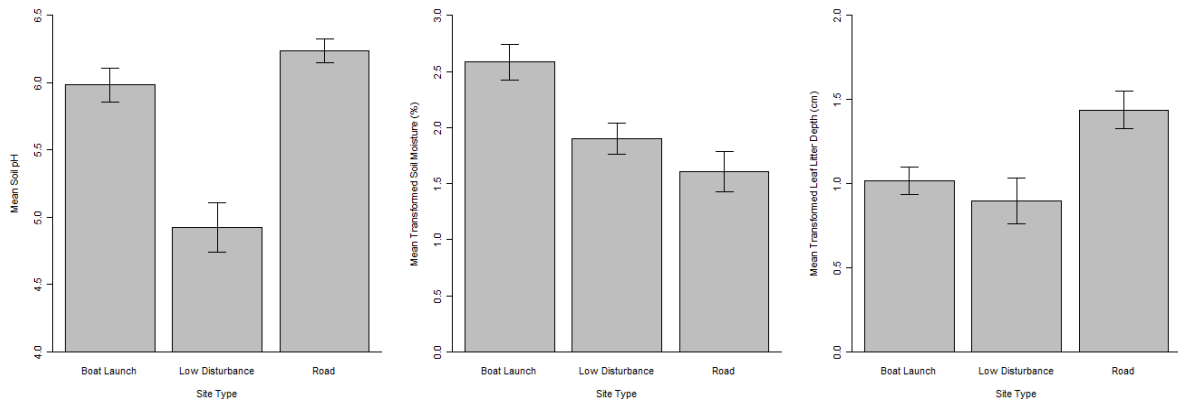

Figure 5. Mean (± se) soil pH, square-root transformed soil moisture (%), and square-root transformed leaf litter depth (cm) by site type ($n = 69$).

grass, lichen, litter, and forbs cover percentages; Table 5) used to investigate the occurrence of earthworms. Path distance was not included in the PCA due to its low communality of 0.56. Low communalities indicate weak correlations among variables (Mertler and Vannatta 2010). Two principal components emerged from the PCA that together accounted for about 66% of the total variability in the original variables. There were a large number (53%) of sample sites where the residuals (the difference between the empirical and the reproduced correlations) exceeded 0.05. This suggested that we include additional components (Mertler and Vannatta 2010). However, adding a third component neither changed the number of residuals exceeding 0.05 nor greatly improved the model, so only two components were retained. The first component supported a high pH loading and accounted for about 35% of the variability with positive loadings from soil pH and percentages of moss, grass, and lichen cover. The second component supported a high leaf litter loading and accounted for 27% of the variability in the original variables with high positive loadings from percentage of litter cover, leaf litter depth, and soil moisture (Table 6). The two components generated from the PCA,

Table 5: Correlation matrix for variables used in principal component analysis.

	Mean litter percentage	Mean moss percentage	Mean grass percentage	Mean forbs percentage	Mean lichen percentage	Mean soil pH	Mean soil moisture	Mean leaf litter depth
Mean litter percentage	1	-	-	-	-	-	-	-
Mean moss percentage	-0.40**	1	-	-	-	-	-	-
Mean grass percentage	-0.58**	-0.48**	1	-	-	-	-	-
Mean forbs percentage	-0.13	0.26	-0.30*	1	-	-	-	-
Mean lichen percentage	-0.22	0.50**	-0.29*	0.00	1	-	-	-
Mean soil pH	0.12	-0.64**	0.50**	-0.34**	-0.48**	1	-	-
Mean soil moisture	-0.38**	-0.18	0.57**	-0.18	-0.30*	0.32*	1	-
Mean leaf litter depth	0.65**	-0.45**	-0.20	-0.20	-0.26*	0.05	-0.34**	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

principally a pH and leaf litter pair, were used with the path distance and forbs percent cover (not accounted for in the PCA) in logistic regression to determine the variables that best predicted earthworm presence and absence. Of these four variables used in binary logistic regression

Table 6: Factor analysis loadings for components: ($n = 63$)

Variable	Component		
	1	2	3
Moss percent cover	-0.825	-0.353	-0.075
Soil pH	0.830	0.016	-0.009
Grass percent cover	0.720	-0.567	-0.089
Lichen percent cover	-0.666	-0.190	-0.540
Litter percent cover	0.084	0.929	0.005
Leaf litter depth	0.220	0.825	-0.090
Soil moisture	0.532	-0.589	0.084
Forbs percent cover	-0.450	-0.072	0.835

Table 7: Variables attempted in binary logistic regression ($n = 63$)

Variable	Score	Sig.
Path distance	11.002	0.001
Forbs percent cover	3.734	0.054
High pH component	0.115	0.735
High leaf litter component	0.083	0.773

(Table 7), only path distance was significant at predicting earthworm occurrence ($x^2 =$ 13.74, p < 0.001, $n = 68$). The model was correct overall in 74% of the sites. The logistic regression performed better predicting where worms were present than where absent. The model correctly predicted occurrence of earthworms in 89% of the sites with earthworms and in 38% of the sites without earthworms. The best fit binary logistic equation suggested that at about 5.5 km from the road there was a 50% probability of finding earthworms (Figure 6).

Discussion:

Within the Kenai National Wildlife Refuge (KNWR) exotic earthworms were surveyed at road, boat launch, and low impact (*i.e.,* remote from road) sites to identify the species composition, distribution, and possible limiting factors for earthworms. Exotic

earthworms were found to inhabit 90% of road corridors and 80% of boat launch sites, but only 50% of low impact sites. These results suggest that human traffic influences earthworm presence in the KNWR. Likewise, Cameron and Bayne (2007) in Alberta, Canada, found a higher probability of earthworm occurrence at boat launches and
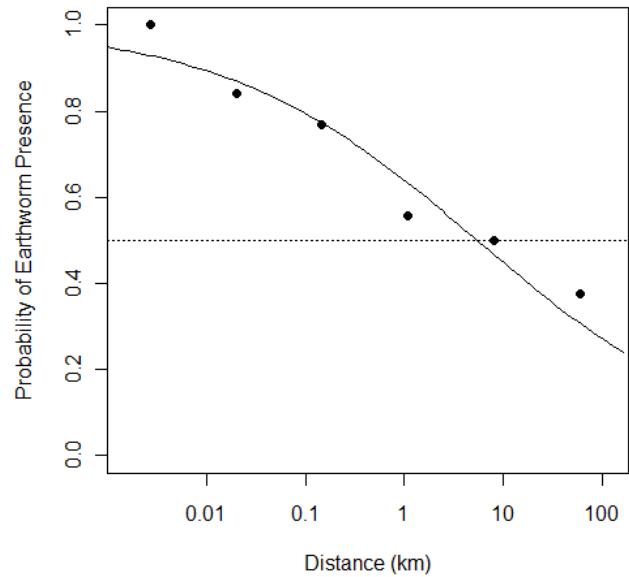

Figure 6: Logistic regression predicting probability of earthworm occurrence from site distance away from roads. Dotted line shows 50% probability of occurrence.

roads compared to forest interiors and remote shorelines. Gundale et al. (2005) in Michigan, found exotic earthworms at all non-wilderness sites (fishing, timber harvest, road), but at only 50% of wilderness sites (designated Wilderness with no history of logging). The road system in the KNWR, while relatively free of roads compared to parks and refuges outside Alaska, is fairly extensive compared to other Alaskan conservation units (for example the 78,050 km^2 Arctic National Wildlife Refuge has no roads). Within the KNWR, the paved Sterling Highway (35 km) and gravel Skilak Lake Road (31 km) bisect the Refuge. The Sterling Highway provides the only road access to towns on the western and southern parts of the Kenai Peninsula, with annual traffic volume exceeding 1.7 million vehicles in 2010 (Witt et al. 2011). Heavily used, especially in summer by tourists and residents alike, these roads provide access to the Refuge and surrounding lands for hunting, fishing, hiking, skiing, and snow-mobiling (Berg 2000). In addition, the gravel Swanson River Road (21 km) and the gravel Swan Lake Road (21 km) are

heavily used in the northern part of the Refuge and provide access to 2,900 km of seismic lines (in place as early as the 1950s; FWS, 2010), and active oil fields. Road age has been linked to earthworm presence. In northern Alberta, Cameron and Bayne (2009) found that older road corridors (average age of 46 years) were significantly more likely to have earthworms than younger ones. The few roads present with the KNWR were built in the 1940s and 50s, suggesting that these 60--70 year old roads have likely contributed greatly to the distribution of exotic earthworms within the KNWR.

We found *Dendrobaena octaedra* as the most widespread (adults at 47% of 70 study sites) and abundant species (10.1 ± 2.2 ind./m^2) at our study sites. This species, along with *D. attemsi* and *Dendrodrilus rubidus* are most likely introduced and spread by vehicles, because of their small size and epigeic habits (i.e., inhabit near-surface of the leaf litter), increasing their likelihood of dispersal by human activities. In contrast, *Lumbricus terrestris* is an anecic species that lives deep in the soil (Hale 2005, Suarez et al. 2006, Addison 2009). *L. terrestris* which is a common species used for fishing bait was found only at three boat launches within 5 km of one another along the Swan Lake Road (Figure 1), suggesting direct bait abandonment or soil discard with *L. terrestris* cocoons as invasion events, although fishing bait can contain other species as well (*Aporrectodea* species, *D. octaedra*, *Dd. rubidus*; Tiunov et al 2006).

Dendrobaena octaedra was the most abundant and dispersed species found in our study. This is not surprising as *D. octaedra* is a prominent invader throughout North America, often both the most widespread and densest exotic earthworm (Cameron et al. 2007, Holdsworth et al. 2007). *Dendrodrilus rubidus* was found at three locations geographically distant from one another (Figure 1) suggesting different vectors for

introduction. *Dendrodrilus rubidus* is common in coniferous forests in its native European and introduced North American range (Addison, 2009). Like *D. octaedra*, it is tolerant of both acidic conditions and frost and as an epigeic species likely impacts the forest floor ecosystems less than anecic species (Addison, 2009). *Dendrodrilus rubidus* is a fairly common earthworm that appears to be present more in northern hardwood and coniferous forests throughout Alaska and Canada (Cameron et al. 2007, Addison, 2009, Costello et al. 2011) than hardwood forests in the Midwest and other areas of the contiguous United States (Hale 2005, Suarez et al. 2006, Holdsworth et al. 2007). We believe this study provides the first record of *D. attemsi* in Alaska (Bower 2010). Found at only two sites in the KNWR near Lake Tustumena, *D. attemsi* was first described in Austria (Michaelsen 1902) and while it is widely distributed in Europe, it is a fairly rare earthworm, suited to acidic, organic rich soils (Rota and Erséus 1997). Limited information on the distribution of *D. attemsi* in North America suggests its earliest recorded presence in the United States in an Oregon greenhouse in 1981 and under bark mulch in a garden in Washington a few years later (Fender 1982, Fender 1985). Specimens have also been collected more recently in the Olympic National Forest, Washington (Lund et al. 2008). In Canada, this species has been noted only in British Columbia (Marshall and Fender 2007). Taken together, these reports suggest that *D. attemsi* is a recent and primarily northwestern exotic earthworm in North America. The fact that it was found only near Lake Tustumena in this study suggest that a single vector was likely responsible for both introductions.

In this study, the single-most important factor determining earthworm occurrence appeared to be distance to roads. Half of our low impact (> 600 m from any road or

facility and 50 m from any trail or river) sites contained earthworms. This is relatively

higher than Cameron and Bayne (2007), who noted 8--35% of their remote transects

(300-500 m in the forest interior) contained earthworms, but similar to Gundale et al.

(2005) who found 50% of wilderness areas without earthworms. The difference between

occurrence patterns in KNWR and the above studies, whereby sites far from roads still

supported earthworms, may be due to other access methods, such as boat and amphibious

float plane, into more remote regions of the Refuge. Holdsworth et al. (2007b) also found

that of all habitat and distance variables, distance to roads was the only significant

predictor of earthworm occurrence in a Wisconsin hardwood forest for most earthworm

groups. They found no significant predictors for the *Dendrobaena* taxonomic group;

however, they found the odds of encountering the *Dendrobaena* assemblage actually

increased by 0.4 times with each additional 100 m traveled away from the nearest road.

Holdsworth et al. (2007b) note that *Dendrobaena* species are early colonizers among

earthworm assemblages, suggesting that the KNWR, too, may be in the early stages of

earthworm colonization. This is also supported by our finding that about 20% of sites had

only juveniles, most likely *D. octaedra*, as would be expected in an expanding

population.

  There are other anthropogenic influences that likely contribute to earthworm

presence within the KNWR, especially in more remote areas. Many of the vectors in the

less accessible parts of the Refuge are not easily identified. The spatially clustered

distribution of some of the species suggests several introductions by potentially multiple

vectors. As mentioned above, individuals of *D. attemsi* were found near Lake Tustumena,

where historic mining activities, a commercial fish processing facility, and several

historic cabins, but no seismic exploration, are present. *Lumbricus* spp. were found only at three boat launches relatively near each other along the Swan Lake Road which suggest bait abandonment as the mode of introduction. *Dendrobaena octaedra* were found throughout the entire Refuge, most likely introduced by road construction, but also possibly by seismic exploration, fire suppression activities, and mechanical tree crushing in the northern part of the Refuge during the 1970s. There are 2,900 km of seismic lines, mostly in the northern part of the Refuge (Figure 7). These lines have been in place since as early as the 1950s, and many remain visible on the landscape today as animal, hiking, and snowmobile trails, as well as illegal access routes for ATVs (FWS, 2010). There have also been numerous prescribed and wild fires within the KNWR (Figure 8), together with associated control and extinguish efforts using heavy equipment, such as bulldozers, that provide additional opportunities for earthworm establishment. There are also non-anthropogenic vectors that can spread earthworms such as birds (D. Saltmarsh, *pers. obs.*) and streams. In southeast Alaska Costello et al. (2010) found earthworms likely use streams for dispersal and showed several taxa could survive at least six days submerged in a stream.

Factors such as soil pH likely also limit earthworm distribution. Most earthworms are found in a soil pH of $5 - 7.4$ (Addison 2009). While earthworms were found in the present study at sites with slightly higher pH (5.74 ± 0.13, $n = 48$ sites) compared to sites without earthworms (5.32 ± 0.23, $n = 21$) and found less frequently at low impact sites, where pH tended to be lower overall (4.92 ± 0.18, $n = 29$), the distribution observed was most likely due to the distance from human impacts rather than pH. The average pH of the low impact sites was significantly different from the other site types, likely due to the

high number of low impact sites dominated by conifers (23% of 30 sites) compared to boat launch (10% of 20 sites) and road (5% of 20 sites) sites. Addison (2009) cites references documenting earthworms under fairly acidic conditions: *D. octaedra* have been found in areas of Canada with a pH as low as 2.8--3.6; *Dd. rubidus* and *L. terrestris* have been recorded in areas with pH of 3.0--3.4, suggesting that even KNWR low impact sites were well within the range of tolerance for both species.

Figure 7. Historic seismic line activity within the KNWR.

Earthworm densities (0--272 ind./m^2) showed substantial variation, with a mean abundance of earthworms at sites with worms of 41 ind./m^2, a value comparable to other studies, suggesting that the species may not be at the limit of its range. Cameron and Bayne (2007) found earthworm densities along transects in Alberta to be 0--335 ind./m^2 and overall averages 2--41 ind./m^2. Gonzalez et al. (2003) found average densities in an aspen forest in Colorado to be

Figure 8. Historic fire activity within the KNWR.

44.4 ind./m^2. Where present, earthworm densities were highest at boat launches and lowest at road sites, although the mean earthworm density did not differ significantly
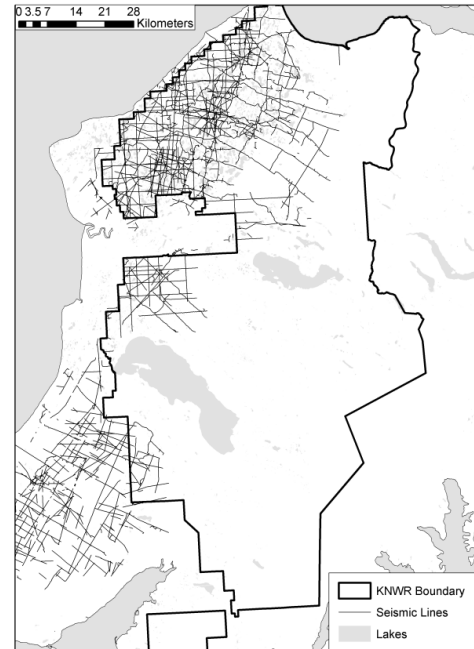
between impact types. Even though low impact sites had about five more earthworms per meter squared than road sites, low impact sites had almost three times the variability in density than roads, supporting a hypothesis of ongoing invasion in low impact sites. Boat launches had the highest density of earthworms (50.0 ± 9.0 ind./m^2) likely due to introduction from both roads and bait abandonment, as well as close proximity to campgrounds (Cameron et al. 2007). In a laboratory setting Bindesbøl et al. (2007) found that *D. octaedra* reached maturity at 12--16 weeks and under control conditions, the population growth rate of *D. octaedra* was ~ 1.12 individuals per week. A study done on *Eisenia foetida* (a common composting worm) found earthworms matured around six weeks and at low population densities (about 5 adults/m^2) produced about five cocoons per worm per week (Hartenstein et al. 1979). This suggests that new populations of earthworms can grow and reproduce quickly, potentially producing more than one generation in a year.

Earthworm impacts on the forest floor vary by feeding strategy (Gundale et al. 2005, Hale et al. 2005). Typically epigeic species such as *D. octaedra* physically disrupt the organic layer by consuming and mixing the top layers and producing a homogeneous, coarse form of organic forest litter. Anecic earthworms such as *L. terrestris* remove litter from the surface, pull it down into the mineral layer and deposit casts of mixed organic and mineral material on the soil surface (Addison et al. 2009). In Minnesota Hale et al. (2005) found that the *Dendrobaena* group alone did not remove the forest floor or change other soil parameters, while *L. terrestris* resulted in the complete removal of surface litter and the lowest percentage of organic matter in the A horizon. They also found that fine root density, total fine root biomass, and nutrient availability were lower in *L. terrestris*

dominated areas compared to others (Hale et al. 2005). These observations in other systems suggest that *D. octaedra* has a lesser impact on forest floor ecology than *L. terrestris,* so ecological impacts may not be apparent within the *D. octaedra* dominated KNWR but the Swan Lake Road should be monitored for effects of *Lumbricus.*

In temperate studies earthworm invasions appear to follow a predictable succesional sequence, beginning with early colonization by epigeic species, such as *D. octaedra,* and epi-endogeic species, like *L. rubellus.* These species are followed by colonization by endogeic and anecic species like *L. terrestris* (Hale et al. 2005, Tiunov et al. 2006, Addison 2009). Gundale et al. (2005) confirmed this sequence in Michigan where they found communities consisting of just one or two species that were almost exclusively composed of *D. octaedra* and *L. rubellus*. This was similarly observed by Suarez et al. (2006) in New York where the edge of earthworm distribution was dominated by *L. rubellus*, followed by communities dominated by *L. terrestris*. This sequence can largely be explained by the differences in species traits such as reproductive strategy, fecundity, cold tolerance, and colonization rates. *D. octaedra* is partheogenic, has high cocoon production (Dymond et al. 1997), is extremely frost tolerant and can withstand over-winter freezing in all stages of development down to at least -14 ℃. Together, these traits in *D. octaedra* aid in its success as an initial invader (Holmstrup 2002, Holmstrup et al. 2007). Thus we expect this species to spread most widely in Alaska.

In Alaska, the primary factors affecting current distributions of earthworms appear to be the soil and litter properties of habitats across the region, although we were unable to detect such an effect on the Kenai Peninsula. Nevertheless, the distribution of

permafrost and cold winter temperatures, as well as soil moisture and pH, likely limit

their potential Alaskan distribution. Where earthworms can survive, historic and current

human activity and land use practices, and the composition of particular source

populations, likely determine earthworm presence. Both *D. octaedra* and *Dd. rubidus* are

parthenogenic, frost-hardy species. These traits combined with their ability to tolerate

acidic soils and exploit poor litter quality, likely contribute to their success in colonizing

large areas. The parthenogenic trait may facilitate reproduction at very low densities,

characteristic of extreme habitats and rare dispersal events, where a single individual can

establish an entire population (Tiunov et al. 2006). The small body size of these species

also facilitates spread by vectors such as tires more often than *Lumbricus* and other

anecic species. Given its wide distribution on the KNWR and its particular ecological

traits, *D. octaedra* will likely be able to colonize large areas of permafrost-free Alaska, an

expanding region as permafrost continue to melt with a warming climate (Osterkamp

2005). While *D. octaedra* has limited impacts compared to other exotic earthworm

species, its presence could signal a larger community invasion if Alaska follows the same

colonization sequence of seen elsewhere.

Conclusions:

Our study describes the current distribution and abundance of exotic earthworms

within the Kenai National Wildlife Refuge at road, boat launch, and low impact, more

remote sites. Of the four earthworm species we found, *D. octaedra* was the most

widespread and abundant, likely due to dispersal by road-based vectors, but also by less

obvious vectors such as fire management activities, seismic exploration, streams, and trail

and transmission line construction within the KNWR, and by boat and plane access. The frost and acidic soil tolerances, coupled with parthenogenic reproduction and ecological preferences of *D. octaedra* and *Dd. Rubidus,* make these two species better suited and more likely to spread throughout Alaska than other species, especially as temperatures warm and more habitats become available. Because *Lumbricus* species are more damaging to native ecosystems, we suggest that this species be monitored both in the KNWR and elsewhere in southcentral Alaska when found to be present.

There have been limited studies on exotic earthworms in Alaska, and in northern climates in North America in general. As there are no effective strategies for removing exotic earthworms once they are established, preventing invasion is vital to reducing their overall impact. This study indicates that minimizing road construction and bait use would best slow the spread of exotic earthworms in Alaska. The next logical research step on the KNWR, and elsewhere in Alaska, would be to estimate the effects of exotic earthworms found there by comparing ecosystems with and without exotic earthworms. Results of such a study would guide the urgency of further research identifying the species assemblages and distributions over a broader area and delimiting the northern distribution and temperature limits of exotic earthworms in Alaska.

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